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Interfacial and emulsifying properties of mealworm protein at the oil/water interface

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ABSTRACT

The increasing global population and consumer demand for protein will render the provision of protein a serious future challenge. The lower environmental impact of insect farming makes the consumption of insects an appealing solution, although consumers in developed countries often respond to the idea of eating insects with disgust. One approach to accustom consumers to insects as part of their diet is through the application of functional insect extracts as food ingredients. Here, the interfacial and emulsion properties of protein extracted from *Tenebrio molitor* (MP) was investigated in comparison to commercial whey protein (WP). The MP showed higher interfacial activity and faster adsorption kinetics at the oil/water interface. The mean droplet size of high shear processed oil-in-water (o/w) emulsions (20% w/w oil) stabilised with MP assumed a process limited value at the lowest protein concentration, included in this study, of 0.44% w/w based on aqueous phase. Stepwise increase in protein concentration to 0.88%, 1.75% and 2.63% revealed, in the case of WP stabilised emulsions, that the same process limited droplet diameter was reached at 1.75% protein addition. With a view to potential future application of MP as a food emulsifier MP stabilised emulsions were exposed to common formulation and process conditions such as varied pH, salt, heat, chilling and freezing. Except for flocculation after heating to 90 °C and at pH close to the isoelectric point (IEP) of the MP, the microstructure of the emulsions remained unchanged. MP shows promise as a food emulsifier and represents a vehicle for the introduction of insect protein into the diet of societies not accustomed to eating insects.

1. Introduction

The demand for animal derived protein is expected to double by 2050 (van Huis et al., 2013) driven by increasing global population, urbanisation, prosperity as well as the growing importance of protein in the diet in developed and developing countries. A proportion of this demand can be met by increasing both the land and cropping intensity currently used for production. However, the International Livestock Research Institution predicts that at least 70% of this demand must be met by innovative technologies and novel sources of protein (Armstrong, 2009).

One of the novel sources that could help meet future protein demands is insects. With protein contents reported between 35 and 61% (Rumpold & Schluter, 2013) many species of insects are richer in protein than beans (23.5% protein), lentils (26.7% protein) and soybeans (41.1% protein) (Zielinska, Baraniak, Karas, Rybczynska, & Jakubczyk, 2015). Based on an enzymic *in-vitro* assay, the protein digestibility of a selection of edible Mexican insects has been reported to range between 77 and 98% (Ramos-Elorduy et al., 1997). This is higher than for some vegetable based proteins and for some species only slightly lower than values reported for animal protein sources (egg 95%, beef 98%, casein 99%) (Mlcek, Rop, Borkovcova, & Bednarova, 2014).

As well as being a nutritionally valuable source of protein, the commercial practice of farming insects for human consumption, already in place in China, Thailand, Vietnam and Lao People's Democratic Republic (van Huis et al., 2013), is often considered to have a lower environmental impact than that of beef, pork or chicken farming (Jäch, 2003; van Huis et al., 2013). There are several reasons for this including high fecundity with some insects laying around 1500 eggs per month (Nakagaki & Defoliart, 1991), poikilothermic (cold-blooded) nature meaning no energy is required to maintain body temperature (Halloran, Roos, Eilenberg, Cerutti, & Bruun, 2016) and omnivorous nature allowing use of organic side streams as feedstock whilst also having a higher feed conversion

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rate than pigs and cattle (Oonincx & de Boer, 2012). In addition, the land requirement is significantly lower, for example mealworm farming requires only 43% of the land required to produce one kg of milk protein and only 10% of the land used for production of one kg of beef protein (Oonincx & de Boer, 2012).

However, insects do not currently significantly contribute to the human diet in developed countries, but examples do exist where their contribution is acceptable, such as in the production of honey and Carmine E120 (a red food dye extracted from the female cochineal insect used in yoghurt, confectionery and drinks) (Yi et al., 2013). On the other hand, 1900 edible insect species are consumed regularly as part of the traditional diet of at least 2 million people in Africa, Asia and Latin America (van Huis et al., 2013). In developed, non-insect consuming countries there is a certain amount of disgust and distaste about eating insects. The negative perception has arisen due to a certain degree of food neophobia as insects have never played a substantial role in the diet, a disgust about consumption due to their association with nature and animalness (Hartmann, Shi, Giusto, & Siegrist, 2015; van Huis, 2016), as well as a dislike of the appearance of insects (Cicatiello, De Rosa, Franco, & Lacetera, 2016). Recent consumer studies in the Netherlands, Australia and Germany suggest that introducing "invisible insects" into food products may be a route to enhancing consumer acceptance in non-insect consuming countries (Hartmann & Siegrist, 2016; Hartmann et al., 2015; Lensvelt & Steenbekkers, 2014; Schösler, De Boer, & Boersema, 2012). One particular study asked 1083 consumers in the Netherlands to score the attractiveness of 13 vegetarian meals and found that all photos containing insects were rated negatively. However, the image of a pizza containing processed insect protein was rated more positively than chocolate coated locusts, fried mealworm salad and locust salad, indicating that meals with invisible insects trigger less aversion than visible insects (Schösler et al., 2012). For this reason, the incorporation of insect protein extract as a food ingredient might have greater success in terms of acceptance by consumers and could pave the way for consumption of unprocessed insects.

Proteins ingredients in food products contribute significantly to the physical properties and microstructures through their ability to stabilise foams and form gel and fibrillar structures. Compared to the commonly used grain, oilseed, legume or milk proteins research into the functional properties insect protein is in its infancy. In one study the water soluble protein fractions of 5 insect species, namely *Tenebrio molitor*, *Zophobas morio, Alphitobius diaperinus*, *Acheta domesticus* and *Blaptica dubia*, were evaluated for their foaming and gelation properties (Yi et al., 2013). The heat set gels varied in gel strength and in addition to insect species, insect life stage, diet and habitat were proposed as influencing factors. The foaming ability of the protein extracts was poor with foams having a half-life of less than 6 min. This may have been due to the relatively low protein concentration in the samples (around 1.7% w/v) and the presence of oil (around 0.1% w/v) which usually acts as an antifoam or foam destabilising agent (Yi et al., 2013). Caterpillar species *Cirina forda* and *Imbrasia oyemensis,* after drying and pulverisation, have been shown to have high water and oil adsorption properties and after defatting aqueous foam formation was also possible with *Imbrasia oyemensis* (Akpossan et al., 2015; Omotoso, 2006; Osasona & Olaofe, 2010).

Proteins are surface active molecules and hence are also widely used as emulsifiers in food products. During homogenisation of the immiscible liquids oil and water, proteins reduce the interfacial tension and form protective layers around droplets thereby providing stability against droplet coalescence. Upon adsorption to an interface proteins undergo changes in conformation to maximise favourable interactions with each phase e.g. hydrophobic residues are exposed to the hydrophobic oil phase. The time and extent of conformational changes of the protein will depend on the molecular flexibility of the protein and extent of packing of the protein at the interface (McClements, 2004). Typically, proteins with a lower molecular size, higher surface hydrophobicity, surface charge, solubility and flexibility warrant good emulsifying properties (Sharif et al., 2018). For protein stabilised emulsions the main mechanism preventing coalescence and flocculation is electrostatic repulsion, as the interfacial films typically formed are thin, this means that these emulsions are sensitive to changes in the environment that could reduce the magnitude of electrostatic repulsion such as pH and ionic strength (McClements, 2004).

Similarly to animal and plants, proteins extracted from Caterpillar species *Cirina forda* and *Imbrasia oyemensis,* after drying and pulverisation, have been shown to stabilise oil-in-water (o/w) emulsions (Akpossan et al., 2015; Omotoso, 2006; Osasona & Olaofe, 2010). Building on these studies, the aim of this research was to investigate the emulsifying ability of protein extracted from mealworms. The emulsifying abilities of mealworm protein (MP) were compared with the properties of commercial whey protein (WP) and the stability of these MP stabilised emulsions was assessed when included in typical food emulsion formulations.

2. Materials and methods

2.1. Materials

Ethanol, sodium hydroxide pellets (NaOH), hydrochloric acid (HCL), pH 7 phosphate buffer tablets (containing 44.8% disodium orthophosphate heptahydrate, 28% dipotassium phosphate and 27.2% sodium chloride) and sodium chloride (NaCl) used for mealworm protein preparation and emulsion assays were purchased from Fisher Scientific (Loughborough, UK). Double distilled water with 0.02% sodium azide (Sigma-Aldrich, Dorset, UK), added as an antimicrobial, was used for preparation of the protein solutions. Sunflower oil used as the oil phase for the o/w emulsions was purchased from a local supermarket (Sainsbury's, London, UK). The oil was purified using magnesium silicate (Sigma-Aldrich, Dorset, UK) for interfacial tension measurements. BiPRO whey protein isolate (WP) was kindly donated by Davisco Foods International, Inc. (MN, USA).

2.2. Mealworm protein isolation

Mealworm protein was isolated from lyophilised mealworm powder. Live mealworms, *Tenebrio molitor* 18–26 mm, supplied by Monkfield Nutrition (Royston, UK) were frozen at − 80 °C for 24 h before freeze drying for 48 h to a final moisture content of 7%. The freeze dried mealworms were ground to a fine powder using a coffee grinder (30 g sample, 30 s, De'Longhi KG49, Havant, UK).

The first step of extracting the protein from the mealworm powder comprised the removal of the lipid fraction following a published protocol (Zhao, Vázquez-Gutiérrez, Johansson, Landberg, & Langton, 2016). Mealworm powder (30 g) was dispersed in ethanol (150 g) and incubated for 1 h at 40 °C. The solids were then isolated by filtration (Whatman GF/F, Kent, UK) and a second extraction was carried out on the solids. All traces of the solvent in the defatted mealworm powder were removed by vacuum drying overnight at 40 °C (Weiss Gallenkamp, Loughborough, UK). On a dry mass basis (DM) the lipid fraction accounted for 34 ± 1% which was in line with the 33% reported for their mealworms in the reference followed for the extraction protocol (Zhao et al., 2016).

The protein fraction was extracted from the defatted mealworm powder using alkali extraction and pH based protein precipitation following the method described in the aforementioned publication (Zhao et al., 2016). Briefly, 30 g of defatted mealworm powder was

dispersed in 450 g of 0.25 M NaOH and incubated for 1 h at 40 °C. The sample was then centrifuged at 4 °C and 3370 g for 20 min (J2-21M Induction Drive Centrifuge, Beckman, High Wycombe, UK). The supernatant and gel layer were retained, whilst two further NaOH extractions were carried out on the pellet. The supernatant and gel layers from all three NaOH extractions were combined, and the pH was adjusted to 4.3–4.5 with 2M HCL followed by centrifugation at 4 °C and 2400 g for 15 min (J2-21M Induction Drive Centrifuge, Beckman, High Wycombe, UK). The precipitate was washed twice with distilled water followed by centrifugation at 4 °C and 2400 g for 10 min (J2-21M Induction Drive Centrifuge, Beckman, High Wycombe, UK). The washed protein precipitate was frozen at − 80 °C overnight followed by freeze drying to a moisture content of less than 5%. The crude protein extraction yield was 46 ± 3%, slightly lower than the 57% previously reported by Zhao et al. (2016).

Finally, the fraction of the mealworm protein soluble at pH 7 was isolated, as pH 7 offers maximum solubility of mealworm proteins (Azagoh et al., 2016). The freeze dried mealworm protein was agitated in a pH 7 phosphate buffer overnight, followed by centrifugation at 2700 g for 10 min (Jouan CR3i multifunction Centrifuge, Thermo Fisher Scientific, Massachusetts, USA). The supernatant, containing the solubilised protein, was freeze dried to obtain the final mealworm protein powder (MP), which was stored in a sealed container at room temperature until use. The pH 7 soluble fraction accounted for 64 ± 5% of the extracted protein.

2.3. Protein solution preparation

In order to evaluate the interfacial properties of mealworm protein a control sample of whey protein was used. Solutions were prepared from whey protein powder at concentrations of 0.5%, 1%, 2% and 3%. To account for non-proteinaceous material in both protein samples, their protein content was determined using a nitrogen analyser (Flash EA1112, Thermo Scientific, Massachusetts, USA) and converting the nitrogen content into the protein content using the global factor of 6.25. The protein content of the MP was 52 ± 0.5% (DM) due to the presence of buffer salts while the protein content of the WP was 93 ± 5% (DM). These differences in protein content were accounted for when preparing protein solutions. Protein solutions were therefore prepared at 0.44%, 0.88%, 1.75% and 2.63% (DM) concentrations.

Protein solutions (0.44%, 0.88% 1.75% and 2.63% (DM)) were prepared by dispersing the required amount of protein powder in water containing 0.02% of sodium azide and a pH 7 phosphate buffer tablet to maintain constant pH. The dispersions were stirred at room temperature overnight to ensure complete dissolution of the protein.

2.4. Interfacial tension measurement

A drop shape tensiometer (PAT-1, Sinterface, Berlin, D) was used to measure the interfacial tension of each protein at the oil/water interface. The oil was initially purified to remove naturally present surface active molecules impeding the interpretation of the interfacial tension data. This involved adding magnesium silicate (4%) to the oil followed by stirring at room temperature on a magnetic stirrer for 30 min at moderate speed (600 rpm). The magnesium silicate particles were then separated out by centrifugation at 2700 g for 30 min (Jouan CR3i multifunction Centrifuge, Thermo Fisher Scientific, Massachusetts, USA).

The tensiometer was fitted with a straight capillary of 2 mm outer diameter to generate a pending drop of the protein solution in the purified oil phase contained in a quartz glass cuvette. Drop size was controlled and kept constant by cross-sectional area (26 mm^2). All of the measurements involving proteins were taken in duplicate at 20 °C and values were recorded for 6 h after drop formation. Interfacial tension values are reported as average of the two final recorded values that differed by less than 1 mN/m for each sample. The bare oil/water interface was analysed in duplicate.

2.5. Emulsion assays

2.5.1. Emulsion preparation

O/W emulsions were prepared by mixing 80 g of aqueous pH 7 protein solution (0.44%, 0.88%, 1.75% and 2.63% protein, DM) with 20 g of sunflower oil followed by homogenisation using a high shear overhead mixer (L5M Series fitted with emulsor screen, Silverson, Chesham, UK) operating at 8000 rpm for 2 min. The emulsion samples were prepared in triplicate, stored at 25 °C and periodically analysed for stability as outlined subsequently. Emulsions containing the lowest concentration of MP were also submitted to environmental stress tests including pH, ionic strength and temperature abuse. This emulsion formulation was chosen to save on MP extract since its droplet size and stability was not different to those formulated at higher MP content.

2.5.2. Environmental stress tests

O/W emulsions stabilised with 0.44% protein were prepared and subjected to either pH, salt or temperature stress. The emulsion preparation was as described above, with the exception that protein solutions for samples submitted to pH changes were prepared in the absence of the pH 7 phosphate buffer tablet. The emulsions were then stored at 25 °C for 24 h before pH, ionic strength or temperature stress tests were applied as follows.

The emulsions were pH stress tested by adjusting pH to 2, 4, 6 or 8 by adding either 1M HCl or 1M NaOH under slight agitation on a magnetic stirrer. The influence of ionic strength on emulsion stability was investigated by adding NaCl to final solution concentrations of 130, 230 or 330 mM, the contribution of 80 mM NaCl from the buffer tablets was taken into account. The additional NaCl was added under slight agitation on a magnetic stirrer. Following the pH and salt stress tests, the emulsions were stored at 25 °C for 24 h (48 h after homogenisation) before analysis.

Finally, the influence of temperature on emulsion stability was assessed by placing 15 g of emulsion in 28 mL glass vials and placing the samples in a heated water bath at different temperatures (60, 70, 80 and 90 °C) and holding for 30 min, after which the emulsions were stored at 25 °C for 24 h (48 h after homogenisation) before analysis. Emulsion samples were also chilled in a domestic refrigerator at 4 °C for 24 h or frozen at −20 °C for 24 h. After which the samples were allowed to return to room temperature before analysis (approximately 48 h after homogenisation).

All of the samples, except the chilled and frozen samples, were analysed for a second time 7 days after the stress tests (storage at 25 °C).

2.5.3. Analyses for stability

The stability of the o/w emulsions prepared with MP and WP at different protein concentrations (0.44%, 0.88%, 1.75% and 2.63%, DM), and those prepared at 0.44% MP and exposed to different pH, salt and thermal environments, were assessed at various time points during storage at 25 °C by monitoring changes in microstructure, droplet size distribution and zeta potential.

The microstructure of the emulsions was revealed by microscopy (EVOS f1, AMG, Washington, USA). A drop of sample was placed onto a glass slide, covered with a cover slip and then imaged using appropriate magnification and bright field illumination.

Droplet size distributions of the emulsions were measured with a low angle laser diffraction particle size analyser (LS 13 320,

Beckman Coulter, High Wycombe, UK) fitted with an aqueous dispersion cell (Universal liquid module, LS13 320, Beckman Coulter, High Wycombe, UK). Three independent replicates of each sample were measured at 25 °C. Diffraction data were analysed with the equipment software choosing the MIE theory and refractive indices of 1.333 for the dispersant and 1.464 for the dispersed phase, the absorption value was set to 0. The results are reported as volume based mean diameter ($d_{4,3}$) averaged over the three replicates.

The zeta potential of the emulsions was determined using equipment operating under the principle of photon correlation spectroscopy (Delsa Nano, Beckman Coulter, High Wycombe, UK). Three independent replicates were prepared from each emulsion after diluting with water at a ratio of 1:10 (v/v). The pH of the buffered emulsion samples remained constant upon dilution, whereas the pH of the pH stressed emulsions changed as reported with the results. The zeta potential of each replicate was analysed four times at 20 °C.

2.6. Statistical tests

To test whether statistically significant differences in interfacial tension, mean droplet diameter and zeta potential existed between samples an ANOVA and Tukey's statistical test was carried out. The level of significance was set at $p = 0.05$. The results of the statistical tests are presented in the Figures and Tables by the presence of letters by the data point. Data points that are significantly different at $p < 0.05$ will have different letters.

3. Results and discussion

3.1. Interfacial tension

The interfacial tension of the bare oil/water interface was 29.7 ± 0.6 mN/m, based on duplicate measurement, and remained constant throughout the period of measurement (at 20 °C) which is testimony of the absence of surface active molecules in either phase. On the other hand, as expected, the interfacial tension of the protein laden interfaces decreased over time, see Fig. 1. The data correspond to the lowest protein solution concentration applied (0.44%) and are representative of all concentrations assessed in terms of kinetics.

As often observed the first interfacial tension value for either protein solution was already lower than that of the bare interface due to the fast adsorption of low molecular weight species during droplet formation. The decrease was initially steeper before asymptotically plateauing from about 25 min onwards after the interface was generated. This shape is characteristic for the interfacial tension evolution of a protein laden oil/water interface. It is attributed to a two stage process as a result of the initial fast diffusion of protein to the interface followed by a slower adsorption delayed by electrostatic and steric hindrance (Felix, Romero, Vermant, & Guerrero, 2016; Noskov, 2014). The initially steeper decrease of the data recorded for the MP laden interface suggests the presence of lower molecular weight proteins compared to WP. Indeed, the upper limit of molecular weight range of mealworm larvae protein, extracted using a comparable protocol to this study, has been reported as 76 kDa (Azagoh et al., 2016) compared to at least 18.6% of the molecular mass of WP being larger than 92 kDa (Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014). The more pronounced first stage for the MP system led to the slightly lower interfacial tension of 11.1 mN/m recorded 6 h after droplet formation compared to 12.4 mN/m for the WP system. Increasing the protein concentration in either system did not significantly decrease the value of the interfacial tension, indicating that the lowest concentration applied (0.44%) saturated the interface, with additional protein in other samples not adsorbing to the interface.

The lower interfacial tension plateau of the MP laden interface compared to the WP laden interface is hypothesised to be due to differences in the surface hydrophobicity, surface charge, conformation or flexibility of two of proteins. Surface hydrophobicity has been negatively correlated with interfacial tension, as the increased

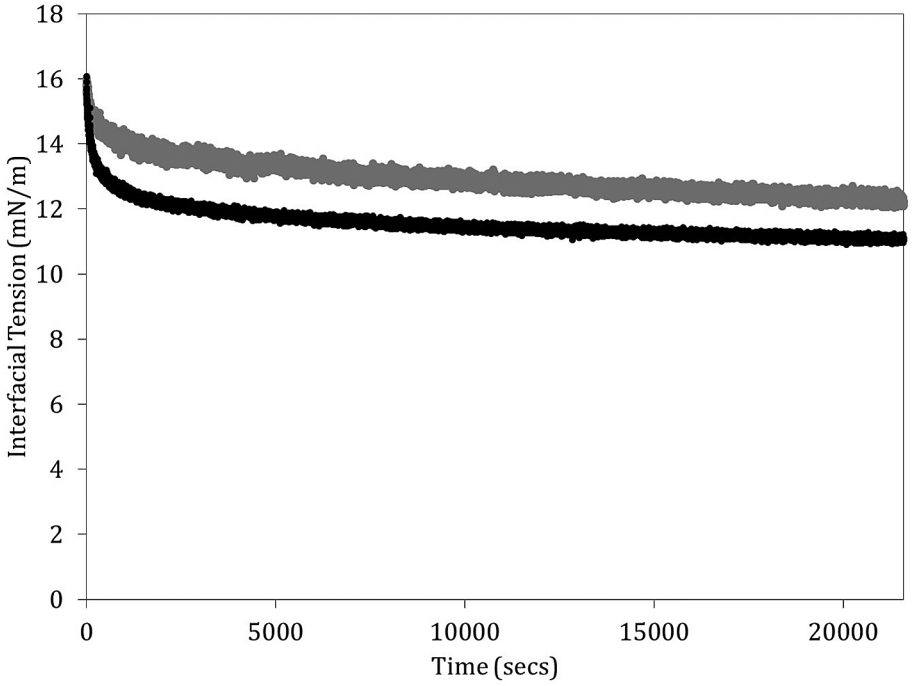

Fig. 1. Interfacial tension between aqueous solutions of 0.44% MP (■) and 0.44% WP (■), respectively, and purified oil (20 °C).

affinity of hydrophobic residues for the oil phase, causes proteins to orientate more readily resulting in a faster and more significant decrease of the interfacial tension (Kato & Nakai, 1980; Keshavarz & Nakai, 1979). The surface hydrophobicity of protein extracted from mealworm has been shown to vary depending on the extraction protocol, however a similar protocol to the protocol used in this study, found mealworm protein to have a high surface hydrophobicity as measured using an ANS fluorescent probe (Azagoh et al., 2016). In contrast, a separate a study, also using ANS fluorescent probe, found whey proteins in their native state to have low hydrophobicity which increased upon unfolding (Moro, Gatti, & Delorenzi, 2001).

In addition, differences in surface conformation also contribute to the differences in interfacial tension as typically rigid globular proteins, such as β-lactoglobulins present in whey proteins, will take longer to undergo conformational changes at the interface compared to more flexible proteins (McClements, 2004). The faster reduction in interfacial tension of mealworm protein could be due to a higher molecular flexibility of the protein compared to whey proteins. A less charged protein will also allow for denser surface coverage and therefore a lower interfacial tension.

The lower interfacial tension of the MP suggests that emulsions prepared with MP at the same processing conditions as emulsions with WP would have smaller droplets as it is known that, at the same given homogenisation conditions, the greater the reduction in interfacial tension created by the adsorption of emulsifiers to an interface, the smaller the emulsion droplets formed (McClements & Gumus, 2016).

3.2. O/W emulsions stabilised with mealworm and whey protein

3.2.1. Impact of protein type on microstructure, droplet size and stability against coalescence

O/W emulsions were prepared from MP and WP solutions at protein concentrations of 0.44, 0.88, 1.75 and 2.63% by homogenisation, using a high shear mixer, in the presence of 20% sunflower oil. Independent of type of protein, the droplet size distributions of the emulsions were monomodal. The results are reported in Fig. 2 as the volume based mean diameter. Fig. 3 shows the microstructure of selected emulsions which was representative across all emulsions and can be described as unflocculated. The micrographs support the numerical data in such that the droplets of the emulsions stabilised with the lowest concentration of WP were larger than the droplets in the emulsions stabilised with the same amount of MP in the system. The difference in volume based mean droplet size between MP and WP stabilised emulsions was statistically significant at this and the next higher concentration of 0.44% and 0.88% ($p < 0.05$). Another observation worth noting is that in contrast to the MP stabilised emulsions, the WP stabilised emulsions showed an impact of protein concentration on droplet size despite the fact that the interfacial tension across the four protein concentration was not different. For the MP 0.44% sufficed to obtain a process limited droplet size spectrum, whereas 1.75% of WP were

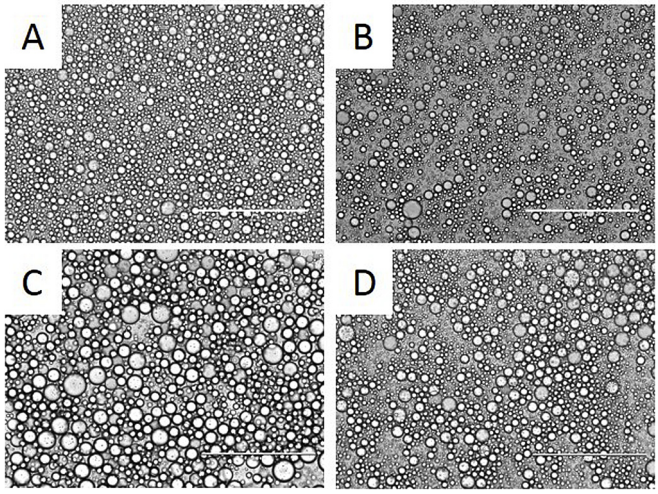

Fig. 3. Light micrographs of o/w emulsions with 20% sunflower oil and stabilised with A) 0.44% MP, B) 2.63% MP, C) 0.44% WP and D) 2.63% WP acquired after one day of storage at 25 °C. Scale bars represent 200 μm.

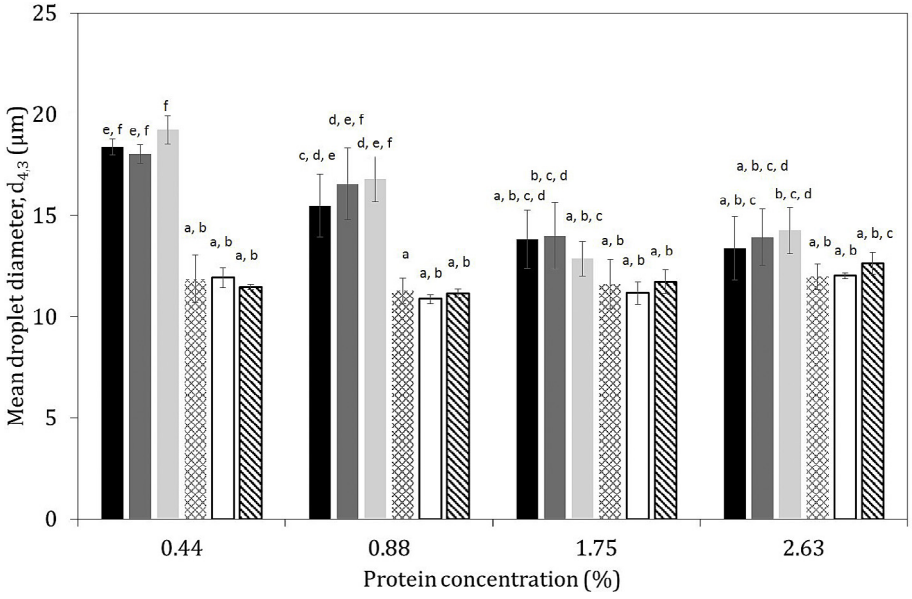

Fig. 2. Mean droplet diameters of emulsions stabilised by varying concentrations of MP and WP acquired day 1, day 7 and 2 months after emulsion formation and storage at 25 °C. The data represented is as follows; (■) WP day 1, (■) WP day 7, (■) WP 2 month, (▩) MP day 1, (□) MP day 7 and (▨) MP 2 month. Error bars correspond to standard deviation of the mean and the presence of different letters indicate a significant difference between samples at $p < 0.05$.

required to attain this situation. The different behaviour is likely to be linked to the assumed higher molecular weight and thus lower diffusivity or the lower density of low molecular weight species in the WP system compared to the MP system. To test this hypothesis acquisition of molecular weight distribution data of the two protein samples are underway.

Stability of the emulsions against droplet coalescence was assessed by acquiring droplet size data at three time points during a 2 month storage period and the data for the volume based mean diameter were included in Fig. 2. There was no significant change ($p < 0.05$) in droplet size and therefore no evidence for droplet coalescence over this storage period in any of the samples.

3.2.2. Impact of protein type on surface charge of emulsion droplets

The surface charge of the protein stabilised droplets was assessed through measuring zeta potential; see Table 1 for the results. The data were acquired following dilution of each emulsion with water and no significant differences were found for the four MP stabilised emulsions. This was not surprising given the interfacial tension and mean droplet size in these four emulsions were similar. All MP stabilised emulsions showed a less negative zeta potential than all of the WP stabilised emulsions as was predicted from the interfacial tension data. In the case of the WP stabilised emulsions, the absolute value of the zeta potential of the emulsion stabilised at the lowest protein concentration was significantly higher ($p < 0.05$) compared to the other three concentrations. Although this is in line with the larger droplets in this emulsion, the emulsion prepared at the next highest WP concentration of 0.88% also had significantly larger droplets compared to the emulsions stabilised at the two highest protein concentrations while the zeta potential was not different. This indicates that 0.88% of WP was close to the concentration where the droplet size spectrum would be process limited.

A zeta potential of −30 mV is often reported as a critical value below which emulsions are seen to flocculate (Grumezescu, 2016) requiring the addition of thickening agents to prevent creaming. Flocculation also tends to promote coalescence. Here, while the absolute value of the zeta potential of the MP stabilised emulsions was less than 30 mV, the emulsions showed no coalescence or flocculation over a 2 month storage period. Long term stability, comparable to and demonstrated a myriad of times for WP stabilised emulsions, can therefore be assumed (Singh, 2011).

These results indicate that the use of MP as an emulsifier is comparable, if not more efficient, than WP offering an alternative source of protein to dairy for food emulsion formulations. With this in mind, the stability of emulsion stabilised by 0.44% MP in systems where the pH, salt concentration and temperature were altered to conditions similar to those experienced during food processing was evaluated.

3.3. Effect of pH on the stability of MP stabilised emulsions

A major consideration for a food based emulsion stabilised by protein is the pH of the system as changes in pH may impart instabilities such as droplet flocculation as the protein charge diminishes around the IEP. While the results presented so far indicate that MP stabilised emulsions are stable against flocculation at less negative zeta potential than the −30 mV critical value that is generally assumed to be required for stability, this data was acquired at pH 7 and cannot be assumed to be indicative of stability closer to zero net charge. Therefore the influence of changing the pH to pH 2, 4, 6 and 8 after emulsion processing was evaluated for the emulsions stabilised with 0.44% MP by assessing microstructure, droplet size and zeta potential 24 h after pH change. Droplet size distribution was re-analysed 8 days after pH alteration. The pHs of these 4 emulsions following dilution with water was 2.9, 4.3, 6.2 and 8.2, respectively. The results of the stability assessment 24 h after pH change are summarised in Fig. 3. Drop size data acquired 6 days later were not significantly different ($p < 0.05$).

Table 1
Surface charge of emulsion droplets stabilised by MP or WP at varying concentrations, measured 1 day after emulsion formation and storage at 25 °C. Different letters indicate a significant difference between samples at $p < 0.05$.

Protein concentration (%)	Zeta potential (mV)	
	MP	WP
0.44	−22.92 ± 0.75 [a]	−33.66 ± 0.70 [b]
0.88	−23.28 ± 0.39 [a]	−30.66 ± 1.70 [c]
1.75	−23.01 ± 0.91 [a]	−31.28 ± 1.45 [c]
2.63	−22.50 ± 1.49 [a]	−30.94 ± 0.96 [c]

The micrographs included in Fig. 4 show evidence of droplet flocculation around pH 4 which coincides with the lowest net surface charge and largest mean droplet diameter measured across the pH 2–8 range. The mean droplet diameters of the emulsions adjusted to pH 2, 6 and 8 were not significantly different from each other. It is clear from the micrographs though that the size data at pH 4.3 relates to floc size rather than primary droplet diameter. Flocculation is promoted by the low net surface charge, which is indicative of the system being close to the IEP of the protein. The sign of the zeta potential changes from positive to negative between pH 4.3 and pH 6.2. Based on the higher absolute value of the zeta potential at pH 6.2 compared to the value at pH 4.3 it can be assumed that the IEP is closer to the lower of these two pH values. pH induced droplet flocculation has also been reported for emulsions stabilised with non-insect based alternative sources of protein such as tomato seeds (Sarkar, Kamaruddin, Bentley, & Wang, 2016), peas (Liang & Tang, 2013) and lupins (Burgos-Díaz et al., 2016).

Mealworm protein has previously been found to have a pH dependent solubility with minimum solubility at the IEP of the protein (Azagoh et al., 2016). Minimum solubility of a protein results in the minimum effectiveness of the protein as an emulsifier (Tokle & McClements, 2011). In this study, the change in the pH occurred after emulsion stabilisation and from the micrographs shown in Fig. 4 it is evident that there was only a decrease in the repulsive forces between protein films on oil droplets causing flocculation. If the pH change was made before homogenisation it is hypothesised that at pH close to the IEP the poor solubility of mealworm protein could reduce the quantity of protein available to adsorb to an interface impacting the droplet size and stability of emulsion formed.

3.4. Effect of temperature on the stability of MP emulsions

To ensure products are safe for consumption thermal processing as well as low temperature storage are often used in food manufacturing. It is desirable that product quality is not compromised by these processes. The influence of therefore exposing the emulsions stabilised with 0.44% MP to 60, 70, 80 or 90 °C for 30 min or storage at 4 °C or −20 °C for 24 h was evaluated by assessing microstructure, droplet size and zeta potential.

Fig. 5 presents the microstructure before (Fig. 5A) and after temperature processing (Fig. 5B–G). There is little difference in the degree of flocculation and droplet size evident for samples heated up to 80 °C (Fig. 5B–D), chilled (Fig. 5F) or frozen (Fig. 5G). However, heating the emulsion to 90 °C caused an increase in droplet flocculation. An explanation for the heat induced flocculation could be the thermal denaturation and subsequent conformational changes of the adsorbed proteins. Conformational changes of adsorbed protein include the exposure of reactive groups, originally

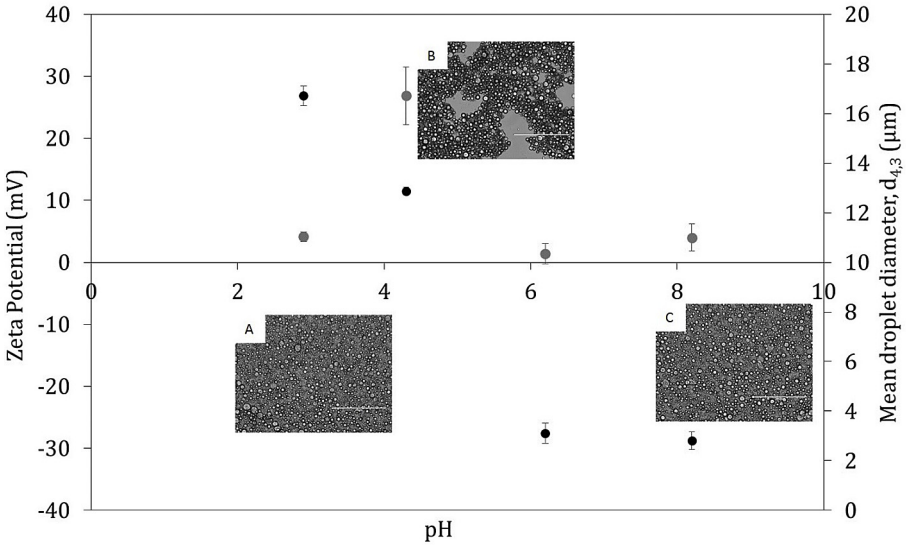

Fig. 4. Effect of pH on zeta potential (egi106BTCJ3SJQ) and mean emulsion droplet diameter (egi106WS1V1GCP) of an o/w emulsion stabilised by 0.44% MP. Data acquired 24 h after altering the pH of the emulsion and are shown versus pH after dilution with water at 1:10. Insets show micrographs of the emulsions following adjustment to (A) pH 2.9, (B) pH 4.3 and (C) pH 8.2. Scale bar in the micrographs equates to 200 μm.

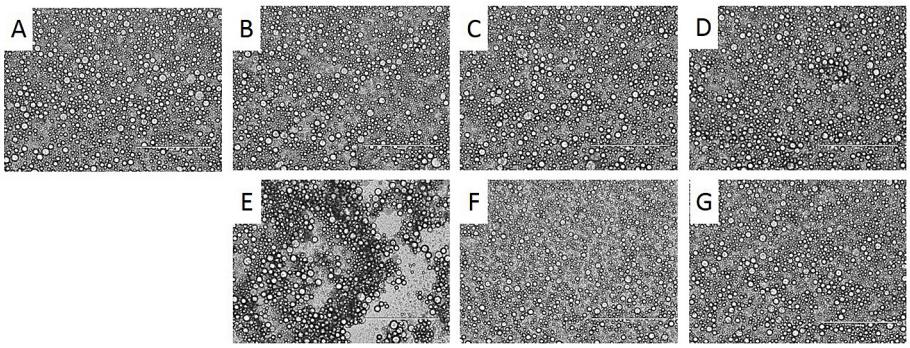

Fig. 5. Light micrographs of o/w emulsions with 20% sunflower oil and stabilised with 0.44% MP subjected to different temperature treatment A) control (untreated) B) 60 °C, C) 70 °C, D) 80 °C, E) 90 °C, F) 4 °C and G) −20 °C after one day of storage. Scale bars represent 200 μm.

located within the protein, leading to an increase in surface hydrophobicity and therefore an increase in protein-protein interactions (McClements, 2004). The temperature stability of whey protein stabilised emulsion has been shown to be dependent on the non-adsorbed protein concentration and heating time, where emulsions with a greater quantity of non-adsorbed protein exhibited more extensive and rapid droplet aggregation when heating above 65 °C and heating at 90 °C for 6–8 min caused an increase in particle size. However, after 130 min of heating the particle size had decreased close to the value of an unheated emulsion (Sliwinski, Roubos, Zoet, Van Boekel, & Wouters, 2003). These results indicate the stability of mealworm protein stabilised emulsion at 90 °C could be increased by reducing the quantity of non-adsorbed protein or increasing the heating time.

Droplet size analysis of the emulsions subjected to the various temperature protocols indicated a significant increase in droplet size after heating to 90 °C compared to the untreated emulsions and emulsions heated to 60 °C and 70 °C, see Fig. 6. In addition, the standard deviation of the mean droplet size for the emulsion heated to 90 °C was considerably larger than for the other samples, which was most likely due to the flocculated microstructure. All emulsions, regardless of the temperature treatment, were stable to coalescence over a period of 7 days of storage at 25 °C. The mean droplet size of the emulsions subjected to chilled storage, analysed after 24 h at 4 °C, was 11.08 μm ± 0.32 μm which was not significantly different to the control samples with a mean droplet diameter of 11.04 μm ± 0.23 μm. In contrast, samples that were frozen after homogenisation had a significantly smaller mean droplet size of 9.16 μm ± 0.31 μm which may be the result of the immediate freezing step preventing initial coalescence of oil droplets to maximise surface saturation with protein.

3.5. Effect of NaCl on the stability of MP emulsions

Salt, routinely added to food products for preservation and flavour enhancement, can be a strong modulator of protein functionality. If the ionic strength of the emulsion formulation is too great the net repulsive forces between protein films, that aid emulsion stability, are overcome and become ineffective at preventing droplet aggregation and flocculation. With this in mind, the influence of NaCl on MP stabilised emulsions was investigated by dissolving varying amounts of NaCl into the emulsion stabilised with 0.44% protein at pH 7 to final salt concentrations of up to 330 mM NaCl. Higher NaCl concentrations were not assessed in this study, as 330 mM NaCl corresponds to 2 g per 100 g of emulsion which would already be labelled as high in salt by UK nutritional guidelines (DOH, 2016).

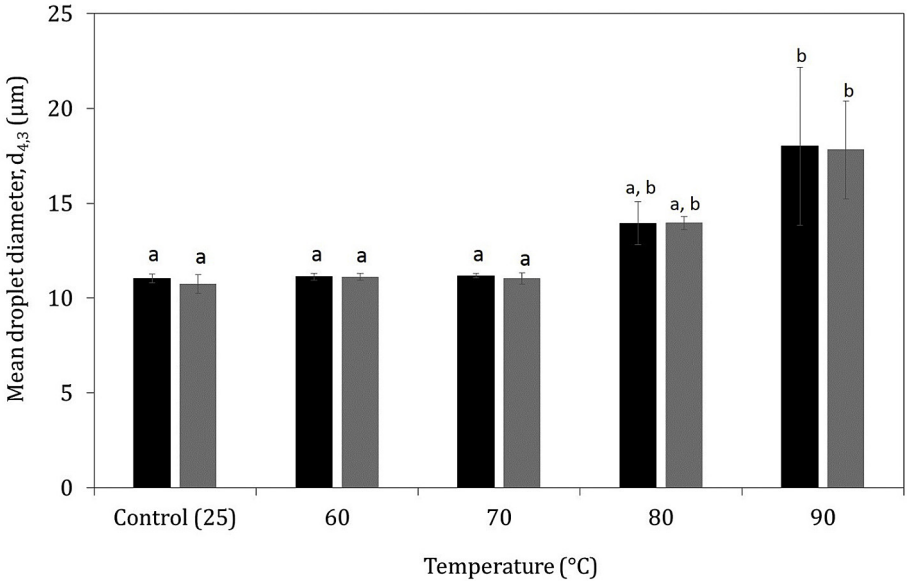

Fig. 6. Mean droplet diameter of o/w emulsions with 20% sunflower oil and stabilised with 0.44% MP subjected to different temperature treatment acquired after 1 day (egi109KW6092NF)and 7 days (egi10QVH9X1B6B) storage. Different letters indicate a significant difference between samples at $p < 0.05$.

All emulsions exhibited good stability to NaCl with no significant change in droplet size compared to the control or over storage, see Fig. 7. The absence of salt induced flocculation in these emulsions was validated by microscopic examination of the microstructure. However, flocculation may occur at higher salt levels based on literature reporting a near net zero zeta potential of mealworm protein at 1 M NaCl. As with the study on pH, the ionic strength of the formulation was altered after homogenisation. Salt addition prior to homogenisation would reduce the number of protein-water interactions due to an increase in salt-water interactions leading to aggregation of the protein (Azagoh et al., 2016). The ability of aggregated proteins to adsorb and stabilise the interface would impact the emulsion microstructure and stability.

4. Conclusions

This research has validated that protein isolated from mealworms can be utilised to stabilise o/w emulsions without significant droplet coalescence for a period of at least 2 months. For food processing applications, mealworm protein stabilised emulsions could be incorporated into formulations containing less than 330 mM sodium chloride as well as products that are chilled, frozen or heated up to 80 °C without altering the microstructure. As with other protein stabilised emulsions, the emulsion droplets flocculated at pH close to the IEP, around pH 4.

In contrast to whey protein, a smaller quantity of mealworm protein was required to generate emulsions of similar

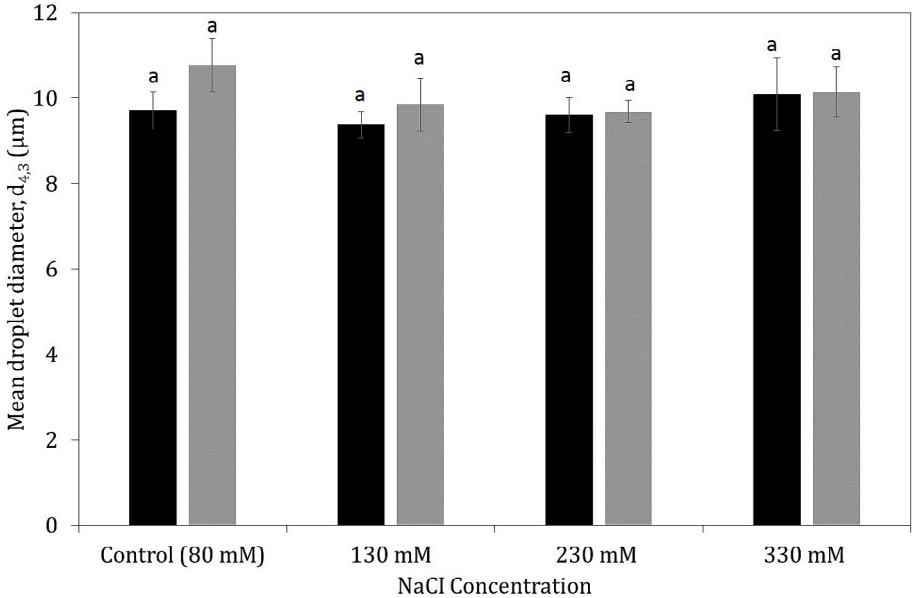

Fig. 7. Mean droplet diameters of o/w emulsions with 20% sunflower oil and stabilised with 0.44% MP at varying NaCL concentrations acquired after 1 day (egi109KW6092NF)and 7 days (egi10QVH9X1B6B) storage. Different letters indicate a significant difference between samples at $p < 0.05$.

microstructure and stability. This is the result of differences in protein molecular weight and most likely interfacial conformation as evidenced by interfacial tension behaviour.

To conclude overall, with regard to microstructure functionality protein extracted from mealworms represents a suitable alternative to current protein based emulsifiers in food formulations. Further research is required to understand the protein structure as well as conformation and adsorption of the protein at interfaces. However, the results are encouraging enough to consider non-microstructure challenges of the use of MP as food ingredient including taste, ethics, consumer acceptance, legal and sustainability.

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